Water relations of pine seedlings in contrasting overstory environments

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ABSTRACT

Overstory conditions influence understory microclimate and resource availability, leading to gradients in evaporative demand and moisture availability that influence seedling water relations. Partial canopies may either reduce seedling moisture stress by ameliorating environmental conditions, or increase moisture stress by reducing soil moisture availability. This study used stable isotope ratios of oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) and mass-based foliar nitrogen concentrations to investigate changes in transpiration (E), stomatal conductance (g_s) and intrinsic water use efficiency (iWUE) of pine seedlings across an overstory gradient from open canopy gap environments to closed canopy forest. Foliar $\delta^{18}O$ increased sharply from basal areas of 0–10 m^2 ha^{-1} in *Pinus banksiana*, *Pinus resinosa*, and *Pinus strobus* seedlings, followed by a more gradual increase with further increases in basal area. Foliar $\delta^{13}C$ followed a similar, but less pronounced pattern in *P. banksiana* and *P. strobus* seedlings, and had no apparent relationship with overstory basal area in *P. resinosa* seedlings. The slope of the $\delta^{18}O$:$\delta^{13}C$ relationship was positive for every species. Foliar nitrogen concentrations were not correlated with overstory basal area. These results suggest seedling E declined as overstory basal area increased due to reductions in g_s, while iWUE increased slightly from open gaps to partial canopy environments. Open gap environments appear to provide sufficient moisture to sustain high leaf-level gas exchange rates in the species we studied, while relatively small increases in overstory basal area apparently promote rapid declines in g_s, leading to greatly reduced seedling water loss and small increases in iWUE.

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1. Introduction

Interest in multi-cohort management in forest types traditionally managed using even-aged techniques has grown in recent years (Guldin, 1996; Lindenmayer and Franklin, 1997; O'Hara, 1998; Palik et al., 2002; Palik and Zasada, 2003; Zenner and Krueger, 2006), but we have a limited understanding of how many of the dominant tree species in these systems respond to the variable overstory environments associated with multi-cohort stand structures. Variation in resource availability and microclimate along structural gradients from canopy gaps and large clearings to closed forest conditions has been well documented (Chen et al., 1995; Palik et al., 1997; McGuire et al., 2001; Heithecker and Halpern, 2007). Variability among overstory conditions is correlated with seedling growth (Palik et al., 1997; McGuire et al., 2001), however, regeneration responses to different overstory environments may be complex and nonlinear in nature (Palik et al., 1997, 2003; Acker et al., 1998). This can make predictions of seedling growth and development in variable overstory environments difficult. Developing an understanding of the functional mechanisms driving regeneration responses among different overstory environments should help us make predictions about patterns of growth and development in those environments.

Light availability is the variable most closely correlated with seedling growth across different overstory environments (Palik et al., 1997; Drever and Lertzman, 2001; McGuire et al., 2001), and much of the research focused on understanding regeneration responses to different overstory environments has focused on relationships between light availability and seedling growth. Changes in soil moisture availability and evaporative demand, however, may also influence seedling development (Dang et al., 1997; Drever and Lertzman, 2001). For example, moisture stress can influence photosynthetic responses to light and other environmental variables (Maier and Tesky, 1992; Parker and Dey, 2008). This implies that understanding how different overstory conditions influence seedling water relations may be an important component of developing a broader understanding of regeneration dynamics in structurally complex forest stands with variable overstory conditions.

Several interacting factors influence seedling water relations by causing both stomatal conductance (g_s) and transpiration (E) to increase as overstory abundance decreases. Soil moisture availability often increases following canopy gap formation or overstory density reductions (Dunlap and Helms, 1983; Aussenac and Granier, 1988; Breda et al., 1995). A number of studies suggest increases in soil moisture availability associated with decreased overstory densities have been shown to reduce stress in seedlings (Dunlap and Helms, 1983; Parker and Dey, 2008; Rodriguez-Calcerrada et al., 2008), which should lead to higher g_s in more open environments. Light availability in the understory also increases as overstory abundance decreases (Wetzel and Burgess, 2001; Palik et al., 2003; Boucher et al., 2007), and g_s generally increases with increasing irradiance (Kaufmann, 1976; Dalton and Messina, 1994; Shimazaki et al., 2007). Thus, changes in both soil moisture and light availability should lead to negative correlations between g_s and overstory abundance. This suggests E should also have a negative relationship with overstory abundance since large changes in g_s also influence E (Pallardy, 2008).

While declines in soil moisture availability associated with increasing overstory density should promote greater seedling moisture stress beneath denser canopies, changes in microclimate associated with different overstory environments also influence E and, therefore, plant moisture stress. Irradiance declines as canopy cover increases, which should decrease air temperatures and increase relative humidity (Dunlap and Helms, 1983; Chen et al., 1995; Aussenac, 2000; Heithecker and Halpern, 2007). As a result, leaf to air vapor pressure deficits (VPD) and potential evapotranspiration generally have an inverse relationship with overstory abundance. The increased VPD associated with open environments can result in higher E, which can increase seedling moisture stress in open environments compared to partial canopy environments (Dalton and Messina, 1994; Aussenac, 2000; Dumais and Prevost, 2008). Excessive moisture stress may lead to stomatal closure (Comstock and Ehleringer, 1984; Dang et al., 1997), which would limit the rate of carbon assimilation (A). In this situation, a partial canopy environment may be beneficial to seedlings because the shaded understory conditions would limit evaporative demand and E (Dalton and Messina, 1994; Aussenac, 2000).

Understanding the relative importance of stomatal and evaporative controls on seedling E in different overstory environments would provide useful insights about patterns of seedling moisture stress that could influence growth and development. Stable isotope ratios of oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) in seedling foliage may offer insights into the physiological and microclimatic factors driving water relations because they are thought to provide integrated signals of leaf-level processes that occurred during tissue formation (Dawson et al., 2002). While direct gas exchange measurements provide quantitative data about instantaneous rates of leaf-level physiological processes, analyses of stable isotopes in plant tissues may offer a qualitative estimate of relative rates of these same processes integrated over one or more growing seasons. Foliar oxygen isotope ratios ($\delta^{18}O$) are influenced by several factors including source water $\delta^{18}O$, evaporative and diffusional processes that occur during transpiration, and oxygen exchange between organic molecules and water. In well-mixed conditions, leaf water enrichment is proportional to $1 - e_a/e_i$, where e_a is water vapor pressure in the atmosphere and e_i is water vapor pressure inside the leaf, and enrichment is positively correlated with ε^k, the kinetic fractionation of water associated with diffusion across the stomata and leaf boundary layer (Craig and Gordon, 1965; Dongmann et al., 1974; Barbour, 2007; Farquhar et al., 2007).

Since E decreases and e_a/e_i increases as VPD decreases and relative humidity increases, leaf water enrichment should be positively correlated with E when differences in E are primarily

Table 1
Expected changes in gas exchange and environmental variables influencing foliar oxygen isotope ratios of tree seedlings as overstory abundance increases and either (a) declines in soil moisture and stomatal conductance drive changes in transpiration or (b) declines in evaporative demand drive changes in transpiration.

Variable[a]	(a)	(b)
g_s	↓	≈
VPD	≈	↓
rH	≈	↑
E	↓	↓
e_a/e_i	≈	↑
ε^k	↑	≈
$\delta^{18}O$	↑	↓

[a] g_s: stomatal conductance; VPD: vapor pressure deficit; rH: relative humidity; E: transpiration; e_a: water vapor pressure in the atmosphere; e_i: water vapor pressure in the leaf; ε^k: kinetic fractionation of water molecules associated with diffusion; $\delta^{18}O$: oxygen isotope ratio.

driven by changes in evaporative demand. The kinetic fractionation of water, however, is negatively correlated with g_s so leaf water enrichment should be negatively correlated with E when differences are primarily driven by changes in g_s (Farquhar et al., 2007). Assuming the isotopic composition of source water is similar, these relationships suggest a positive correlation between foliar $\delta^{18}O$ and E when evaporative demand is the primary driver of changes in E, and a negative correlation between $\delta^{18}O$ and E when stomatal regulation is the primary driver of changes in E (Table 1). Since VPD and g_s generally decrease as overstory abundance increases, we might expect decreasing foliar $\delta^{18}O$ in seedlings growing along a gradient from open to partial canopy environments if VPD is the primary driver of changes in E, or increasing foliar $\delta^{18}O$ across the same gradient if g_s is the primary driver of changes in E.

The carbon isotope ratio ($\delta^{13}C$) of foliage is inversely proportional to the ratio of the leaf's intercellular CO_2 concentration (c_i) to the concentration of CO_2 in the atmosphere (Farquhar et al., 1982). This relationship exists because the carboxylating enzyme Rubisco discriminates against the heavier ^{13}C, and the rate of discrimination declines as c_i declines (Park and Epstein, 1961). The balance between A and g_s is the primary driver of c_i, so there is a positive correlation between foliar $\delta^{13}C$ and intrinsic water use efficiency (iWUE), the ratio of A/g_s (Farquhar et al., 1982, 1989). Since $\delta^{18}O$ may provide information about evaporative processes, and $\delta^{13}C$ provides information about the ratio of A to g_s, several authors have suggested $\delta^{18}O$ and $\delta^{13}C$ can be used together to understand the relative rates of leaf-level gas exchange processes (Saurer et al., 1997; Scheidegger et al., 2000; Barbour et al., 2002). While these studies focused primarily on understanding the sources of variability in $\delta^{13}C$, the dual isotope approach should also be applicable to studies focused on understanding sources of variability in $\delta^{18}O$, particularly across gradients in overstory abundance where the primary environmental factor associated with changes in photosynthesis (light) follows predictable patterns.

In this study, we evaluated relationships between overstory basal area, foliar $\delta^{18}O$, and foliar $\delta^{13}C$ in seedlings of three pine species to better understand trends in water relations across variable overstory environments. We used a dual isotope approach to examine trends in E, g_s, and iWUE. We used this information to partition the relative importance of stomatal and evaporative influences on transpiration in pine seedlings growing across a range of overstory basal areas. Specifically, we believed that foliar $\delta^{18}O$ would either (1) increase as overstory abundance increased as a result of declining g_s and E, or (2) decrease as overstory

abundance increased as a result of declining VPD and E. We further hypothesized that foliar $\delta^{13}C$ would increase as overstory abundance increased if hypothesis 1 about $\delta^{18}O$ was correct because rapid declines in g_s would lead to increasing iWUE, even with the expected decrease in A associated with reduced light levels. If, however, excessive VPD in open environments led to stomatal closure, we would expect a negative relationship between foliar $\delta^{13}C$ and overstory abundance because the combination of high light levels and low g_s in open environments would lead to high iWUE, while reduced light (and A) in shaded environments with lower VPD would lead to lower iWUE.

2. Methods

2.1. Study sites and field methods

This research is part of the Red Pine Retention Study, an experiment testing the impacts of different spatial patterns of overstory retention on vegetation dynamics, productivity, and biological diversity in managed red pine (*Pinus resinosa* Aiton) forests. The Red Pine Retention Study is located on the Chippewa National forest, in north-central Minnesota, USA. The overall study design is described in detail by Palik and Zasada (2003), and Palik et al. (2005), but our study included only a subset of the treatments. The study sites included red pine stands treated with variable retention harvests that created either 0.1 ha (small) or 0.3 ha (large) gaps within a matrix that was thinned to produce residual basal areas of approximately 13 m^2 ha^{-1}. These treatments created considerable within-stand structural variability, such that overstory basal area of 0.08 ha plots within individual stands ranged from 0 m^2 ha^{-1} in gaps to approximately 35 m^2 ha^{-1} in some areas of the matrix between gaps. Treatment stands averaged 16 ha in size, had an even-aged structure with a single canopy stratum, and residual trees averaged 70–80 years of age. Both the small and large gap treatments were replicated in four blocks, so a total of eight stands were included in the study. Treatments were installed in the dormant season of 2002–2003, and 2-year-old, bare root seedlings of jack pine (*Pinus banksiana* Lamb.), red pine, and eastern white pine (*Pinus strobus* L.) were planted at a density of 1300 seedlings ha^{-1} throughout each treatment stand in the spring of 2003. Seedlings were planted in alternating rows with 2.7 m × 2.7 m spacing. Half of each stand received a mechanical understory release treatment in which all shrubs and aspen (*Populus* spp.) regeneration were cut with brush saws every spring following planting. Soils on our study sites were well-drained, coarse-textured sands consisting of mixed, frigid Aquic Udipsamments, mixed, frigid Lamellic Udipsamments, and mixed, frigid Typic Udipsamments, and topographic variability was limited (Richardson, 1997).

Data were collected at 10 study points in each stand. The basal area of all trees greater than 2.5 cm in diameter at 1.4 m in height (dbh) was measured in 0.08 ha circular plots centered on each study point. Samples of previous year's foliage were collected before leaf out in the spring of 2007 from the jack pine, red pine, and white pine seedling closest to each overstory plot center. Foliage was collected throughout the crown of each tree.

Foliar samples were dried at 65 °C for 72 h and pulverized on a ball mill for isotopic and elemental analysis. Foliar nitrogen and $\delta^{13}C$ were analyzed at the Michigan Technological University Forest Ecology Stable Isotope Laboratory using a Costech Elemental Combustion System 4010 connected to a continuous flow isotope ratio mass spectrometer (Deltaplus, Thermo Finnigan, Bremen). Oxygen isotope ratios were measured at the Washington State University Stable Isotope Core Laboratory using a pyrolysis elemental analyzer (TC/EA, ThermoFinnigan, Bremen) connected to a continuous flow isotope ratio mass spectrometer (Delta

PlusXP, Thermofinnigan, Bremen). Whole foliar tissue was used rather than cellulose because a previous study found whole foliage and cellulose provided similar environmental signals in the three species considered here (Powers et al., 2008a).

2.2. Statistical methods

Hierarchical, linear mixed-effects regression models (HLM) were used to evaluate relationships between basal area and the dependent variables $\delta^{18}O$, $\delta^{13}C$, and the mass-based foliar nitrogen concentration (N_{mass}) for each species. Models for N_{mass} were included because gradients in $\delta^{13}C$ have been related to leaf nitrogen for several conifer species (Sparks and Ehleringer, 1997; Duursma and Marshall, 2006). HLM models were fit using every combination of the level one variable basal area, the level two variable overstory treatment (stands consisting of residual trees between either 0.1 ha gaps or 0.3 ha gaps), and the level three variable treatment area (block) including both fixed and random effects for basal area. Individual trees were identified as the sampling unit, grouped by overstory treatment, which was nested within treatment area. Separate models were fit using linear, logarithmic, and quadratic functions of basal area to account for possible curvilinear relationships, and the models were compared using AICC values, a second-order variant of Akaike's Information Criterion developed by Sugiura (1978) to correct for bias associated with a small ratio of sample size to model parameters. Models with the lowest AICC values were generally chosen for further analysis, although models with AICC values within two units of the minimum AICC were sometimes chosen to maximize parsimony or satisfy regression assumptions (Burnham and Anderson, 2002). Initial models were not improved by the addition of an understory release term (nested within overstory treatment), so this variable was not included in any of the final models.

HLM models were also used to evaluate relationships between $\delta^{18}O$ and $\delta^{13}C$ for each species. These models were selected using the same AICC-based process described above. All regression models were constructed using PROC MIXED in SAS version 9.0 (SAS Institute, Cary, NC). Regression assumptions regarding the normality and homogeneity of error variances were assessed using normal probability plots of residuals and plots of residuals against predicted values, respectively.

3. Results

Logarithmic basal area terms always provided better fits than quadratic or linear terms, but some of the final models did not include any basal area term (Table 2). Foliar $\delta^{18}O$ was best modeled using a curvilinear basal area term and treatment area as independent variables. Neither overstory treatment nor any random slope term was included in the final models for $\delta^{18}O$. All species had a significant, logarithmic relationship between foliar $\delta^{18}O$ and overstory basal area ($P < 0.001$ for all). Slope estimates for the logarithmic basal area term were more than 50% higher for jack pine and white pine than red pine. Foliar $\delta^{18}O$ increased relatively rapidly with basal area up to about 10 m^2 ha^{-1}, then increased more gradually with further increases in basal area (Fig. 1).

Foliar $\delta^{13}C$ was best modeled using a curvilinear basal area term, and random intercepts associated with both treatment area and overstory treatment for jack pine and white pine, but varied only by treatment area with no apparent dependence on basal area for red pine (Table 2). No random slope terms were included in the final models for $\delta^{13}C$. Foliar $\delta^{13}C$ had significant, logarithmic relationships with overstory basal area for both jack pine and white pine ($P = 0.024$ and $P = 0.035$, respectively), but no relationship with basal area for red pine. Like $\delta^{18}O$, foliar $\delta^{13}C$ increased

Table 2
Regression coefficients from models predicting foliar carbon isotope ratios ($\delta^{13}C$), oxygen isotope ratios ($\delta^{18}O$), and nitrogen concentrations (N_{mass}) of pine seedlings based on basal area (BA), treatment area, and treatment gap size (SG = 0.1 ha, LG = 0.3 ha). Values in parentheses represent standard error estimates.

Parameter	Pinus banksiana			Pinus resinosa			Pinus strobus		
	$\delta^{13}C$	$\delta^{18}O$	N_{mass}	$\delta^{13}C$	$\delta^{18}O$	N_{mass}	$\delta^{13}C$	$\delta^{18}O$	N_{mass}
Intercept	−29.91 (0.66)	23.08 (1.08)	1.21 (0.09)	−27.73 (0.34)	23.62 (0.46)	0.98 (0.02)	−27.60 (0.47)	22.90 (0.46)	1.10 (0.04)
LN(BA)	0.30 (0.13)	1.36 (0.17)	N/A	N/A	0.86 (0.15)	N/A	0.19 (0.09)	1.43 (0.13)	N/A
Area 1	0.96 (0.64)	1.27 (1.02)	−0.08 (0.09)	0.83 (0.39)	0.41 (0.30)	N/A	1.01 (0.46)	0.20 (0.35)	−0.02 (0.05)
Area 2	−0.07 (0.64)	1.11 (1.02)	−0.09 (0.09)	−0.36 (0.39)	0.03 (0.30)	N/A	−0.47 (0.46)	0.30 (0.35)	−0.01 (0.05)
Area 3	0.32 (0.64)	0.47 (1.02)	−0.07 (0.09)	−0.14 (0.39)	0.01 (0.30)	N/A	−0.09 (0.46)	0.26 (0.35)	−0.05 (0.05)
Area 5	−1.21 (0.64)	−2.85 (1.02)	0.23 (0.09)	−0.33 (0.39)	−0.46 (0.30)	N/A	−0.45 (0.46)	−0.76 (0.35)	0.09 (0.05)
Area 1 LG	0.53 (0.47)	N/A	N/A	N/A	N/A	N/A	0.53 (0.31)	N/A	N/A
Area 1 SG	−0.17 (0.47)	N/A	N/A	N/A	N/A	N/A	−0.24 (0.31)	N/A	N/A
Area 2 LG	−0.12 (0.47)	N/A	N/A	N/A	N/A	N/A	−0.20 (0.31)	N/A	N/A
Area 2 SG	0.10 (0.47)	N/A	N/A	N/A	N/A	N/A	0.07 (0.31)	N/A	N/A
Area 3 LG	0.64 (0.47)	N/A	N/A	N/A	N/A	N/A	−0.01 (0.31)	N/A	N/A
Area 3 SG	−0.52 (0.47)	N/A	N/A	N/A	N/A	N/A	−0.02 (0.31)	N/A	N/A
Area 5 LG	0.09 (0.47)	N/A	N/A	N/A	N/A	N/A	0.20 (0.31)	N/A	N/A
Area 5 SG	−0.55 (0.47)	N/A	N/A	N/A	N/A	N/A	−0.33 (0.31)	N/A	N/A
BA P-value	0.024	<0.001	N/A	N/A	<0.001	N/A	0.035	<0.001	N/A

most rapidly between basal areas of 0–10 m^2 ha^{-1}, and changed relatively little across higher basal areas (Fig. 1). Changes in $\delta^{13}C$ across basal area, however, were small compared to the changes observed for $\delta^{18}O$.

The best models for N_{mass} included only treatment area for jack pine and white pine (Table 2). No N_{mass} models provided better fits than the null (intercept only) for red pine. Foliar nitrogen concentrations showed no apparent relationship to overstory basal area or overstory treatment for any species (Fig. 1). Trees in Area 5 had higher N_{mass} than any other treatment area in jack pine and white pine, but treatment area did not affect red pine N_{mass}.

Foliar $\delta^{18}O$ was significantly, positively correlated with foliar $\delta^{13}C$ for jack pine, red pine, and white pine ($P < 0.001$, $P < 0.001$, and $P = 0.024$, respectively, Fig. 2). The best model for jack pine included foliar $\delta^{13}C$ and a random intercept term associated with treatment area as independent variables, but the best models for red pine and white pine included only foliar $\delta^{13}C$ (Table 3). Overstory treatment was not an important factor for any species. The slope of the $\delta^{18}O:\delta^{13}C$ relationship for jack pine was 70–80% greater than the slope of the $\delta^{18}O:\delta^{13}C$ relationship for red pine or white pine.

4. Discussion

These results suggest seedling E decreases as overstory basal area increases due primarily to decreasing g_s. While the relation-
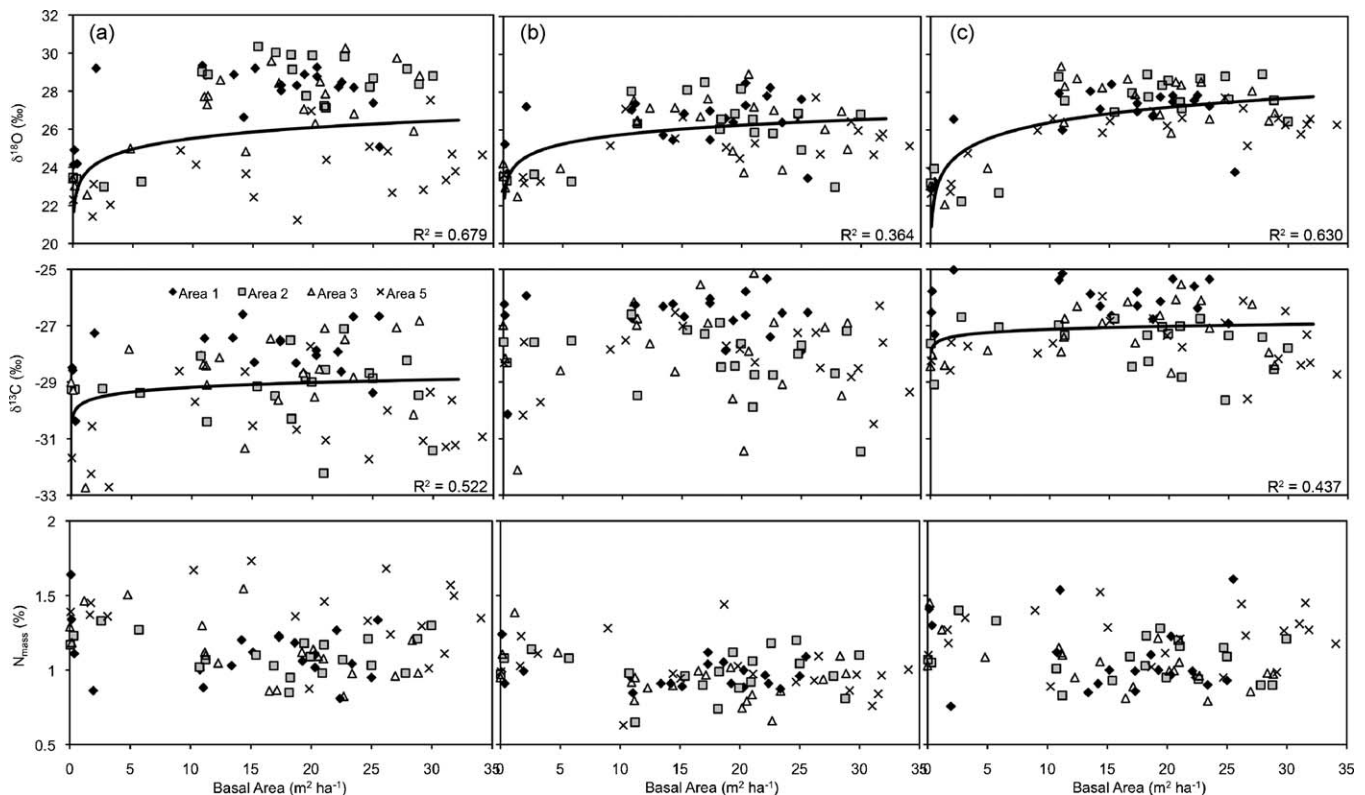
Fig. 1. Trends in foliar carbon isotope ratios ($\delta^{13}C$), foliar oxygen isotope ratios ($\delta^{18}O$), and mass-based foliar nitrogen concentration (N_{mass}) of *Pinus banksiana* (a), *Pinus resinosa* (b), and *Pinus strobus* (c) seedlings in different overstory environments. Data were collected in four treatment blocks (areas 1–3, and 5). Regression lines indicate average relationships with overstory basal area across all blocks, although intercepts associated with individual blocks sometimes varied (see Table 1 for full regression models).
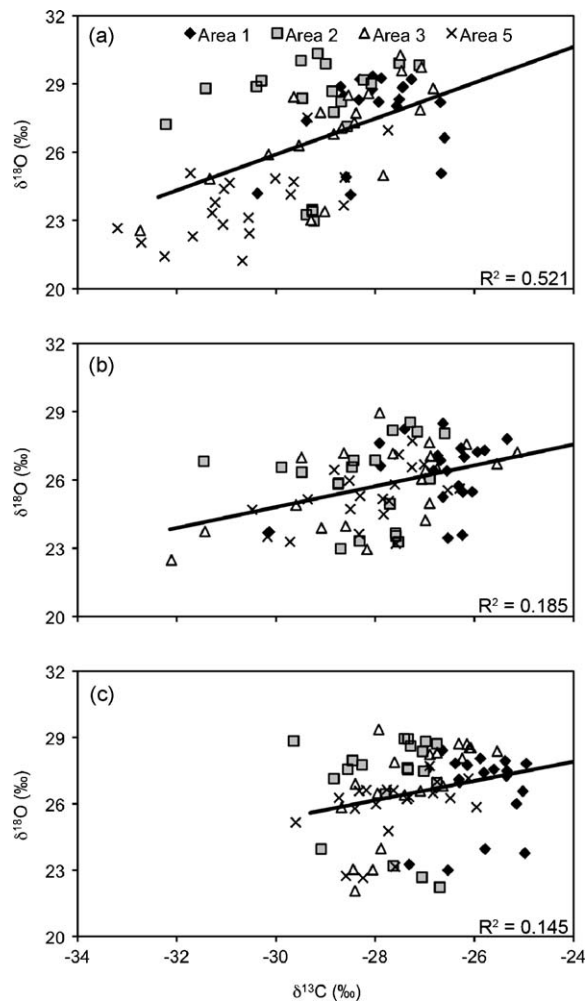
Fig. 2. Relationships between foliar carbon isotope ratios ($\delta^{13}C$) and oxygen isotope ratios ($\delta^{18}O$) of *Pinus banksiana* (a), *Pinus resinosa* (b), and *Pinus strobus* (c) seedlings. Data were collected in four treatment blocks (areas 1–3, and 5). Regression lines indicate average relationships across all blocks, although intercepts associated with individual blocks sometimes varied (see Table 2 for full regression models).

ship we observed between foliar $\delta^{18}O$ and overstory basal area could also be explained by increasing E with increasing basal area due to increasing evaporative demand, this explanation seems unlikely. Although changes in air temperature and relative humidity were not measured, a variety of studies indicate understory air temperatures decline from open canopy gaps to closed canopy environments, while relative humidity increases (Chen et al., 1995; Heithecker and Halpern, 2007). Declines in soil moisture availability and increased plant moisture stress associated with increasing overstory competition, however, are common (Dunlap and Helms, 1983; Aussenac and Granier,

Table 3
Regression models describing the relationship between foliar oxygen isotope ratios ($\delta^{18}O$) and carbon isotope ratios ($\delta^{13}C$) for seedlings of three pine species. Values in parentheses represent standard error estimates.

Parameter	*P. banksiana*	*P. resinosa*	*P. strobus*
Intercept	49.54 (4.76)	38.55 (0.46)	38.37 (5.14)
$\delta^{13}C$	0.79 (0.16)	0.46 (0.12)	0.44 (0.19)
Area 1	0.20 (0.68)	N/A	N/A
Area 2	1.11 (0.66)	N/A	N/A
Area 3	0.10 (0.66)	N/A	N/A
Area 5	−1.41 (0.69)	N/A	N/A
P-Value	<0.001	<0.001	0.024

1988; Breda et al., 1995; Parker and Dey, 2008), and increased moisture stress generally drives g_s down (Maier and Tesky, 1992; Boucher et al., 2007). Light availability also decreases as overstory abundance increases, and irradiance has a positive relationship with g_s (Kaufmann, 1976; Dalton and Messina, 1994; Shimazaki et al., 2007). Thus, the positive relationship between $\delta^{18}O$ and basal area found in this study is most likely caused by a decline in E across the overstory basal area gradient associated with decreased g_s. A previous study of niche partitioning within the gap environments at the same sites found light and soil moisture availability were lower in the partial canopy environment of gap edges than in gap interiors (Powers et al., 2008b), which would lead to decreased g_s and E. While the declines in seedling gas exchange parameters observed from gap centers to gap edges in this previous study were not always statistically significant, they suggest the potential for declines in E across the larger range of overstory environments encompassed within our current study. Foliar $\delta^{13}C$ data provide further support for this conclusion.

Foliar $\delta^{13}C$ increased from open to partial canopy environments in jack and white pines, suggesting an increase in iWUE. The linear form of the relationship between c_i/c_a and $\delta^{13}C$ presented by Farquhar et al. (1982) suggests this could be caused by either an increase in A, a decrease in g_s, or declines in both parameters with larger declines in g_s than A. Light availability declines predictably with increasing leaf area according to the Beer–Lambert law, and basal area (or stem diameter) is highly correlated with leaf area in red pine for both individual trees (Gower and Norman, 1991; Gower et al., 1993) and stands (Deblonde et al., 1994), so there is no clear biological basis for sustained increases in A associated with increasing overstory basal area. Rather, photosynthetic rates are generally higher in more open environments than beneath a denser canopy (Pothier and Prevost, 2002; Parker and Dey, 2008; Rodriguez-Calcerrada et al., 2008). As noted earlier, decreases in g_s are common as overstory abundance increases, so a decline in g_s related to increasing overstory basal area seems the most likely explanation for the observed trends in $\delta^{13}C$. The lack of a significant relationship between foliar $\delta^{13}C$ and overstory basal area in red pine may simply suggest both A and g_s declined at similar rates as overstory basal area increased. While gas exchange measurements are needed to provide direct evidence for changes in g_s, the most likely explanation for the trends in both $\delta^{18}O$ and $\delta^{13}C$ we observed involves large declines in seedling g_s as overstory basal area increases.

The foliar $\delta^{18}O:\delta^{13}C$ relationship provides further evidence for a decline in g_s associated with increasing overstory basal area. Theory suggests the slope of this relationship should be positive if variability in $\delta^{13}C$ is driven by changes in g_s alone, or in combination with parallel changes in A or VPD (Barbour et al., 2002). The positive relationship observed in this study, therefore, suggests the increase in foliar $\delta^{13}C$ associated with basal area was due, at least in part, to a reduction in g_s. Had g_s stayed relatively constant, there would be no expected relationship between $\delta^{18}O$ and $\delta^{13}C$, while if g_s had increased across the overstory gradient the foliar $\delta^{18}O:\delta^{13}C$ should have been negative because changes in g_s would be in the opposite direction of the expected drop in seedling A along the gradient (Barbour et al., 2002).

Several factors other than leaf level gas exchange could contribute to isotopic variability. The analysis presented here assumes increases in foliar $\delta^{13}C$ were correlated with increases in iWUE based on a simple, linear relationship between $\delta^{13}C$ of organic matter and c_i/c_a (Farquhar et al., 1982). Differences in the internal conductance of CO_2 from leaf intercellular spaces to the sites of carboxylation in the chloroplasts, however, could also affect foliar $\delta^{13}C$ if internal conductance does not scale directly with photosynthesis (Warren and Adams, 2006; Seibt et al., 2008). A decrease in resistance to internal CO_2 transfer could contribute to

the increase in seedling δ^{13}C associated with increased basal area. High leaf nitrogen could increase the resistance to CO_2 transfer from leaf intercellular spaces to the site of photosynthesis in the chloroplast (Evans, 1983; Mitchell and Hinckley, 1993), thereby decreasing foliar δ^{13}C. Finally, the isotopic signature of source water influences organic matter δ^{18}O, in addition to any fractionation associated with leaf water enrichment during transpiration (Barbour et al., 2001; Cernusak et al., 2005), which could cause variation in foliar δ^{18}O without any change in evaporative processes in the leaf.

While differences in internal conductance could possibly explain the variability in δ^{13}C across the overstory gradient observed in this study, both foliar δ^{13}C and δ^{18}O data suggest changes in gas exchange parameters consistent with a decrease in g_s, and there was no evidence of variability in N_{mass} associated with overstory basal area that would contribute to differences in internal conductance within a given species. Source water δ^{18}O is determined by the isotopic signature of precipitation, evaporation within the soil, and rooting depth, which were likely similar in this study due to the proximity of the sites to one another and the similarity in both soils and topography among treatment areas. Further, evaporative enrichment should be higher in open environments, leading to greater source water δ^{18}O at low basal areas, which would not explain the positive correlation between foliar δ^{18}O and basal area. Nonetheless, future studies using stable isotopes to understand variability in water relations would benefit from direct gas exchange measurements, micrometeorological measurements and xylem water samples.

These results suggest an abrupt decrease in seedling E and g_s, and slight increase in iWUE as overstory basal area is increased from open gap environments to moderate levels of around $10 \text{ m}^2 \text{ ha}^{-1}$ followed by a much more gradual decline in E and g_s as overstory basal area continues to increase. This finding is consistent with studies reporting increased moisture stress beneath denser canopies (Dunlap and Helms, 1983; Parker and Dey, 2008; Rodriguez-Calcerrada et al., 2008), but conflicts with studies that suggest a partial canopy may improve seedling water relations by limiting evaporative demand (Dalton and Messina, 1994; Aussenac, 2000). In a related study at the same sites, we found predawn xylem pressure potentials were 40–75% lower in the partial overstory environment of gap edges than in open gap environments, suggesting greater moisture stress and lower soil moisture availability in environments with overstory competition than in open gaps (Powers et al., 2008b). These trends in xylem pressure potential suggest differences in soil moisture availability were likely the driving factor behind the trends in E and g_s suggested by isotopic data in this study rather than any change in evaporative demand.

There has been increasing interest in multi-cohort management in the pine forests of northeastern and north-central North America in recent years (e.g. Palik and Zasada, 2003; Zenner and Krueger, 2006), and our findings have several implications for establishing successful regeneration in these systems. First, we found no evidence that VPD was a major driver of transpirational water loss in openings of 0.1–0.3 ha, but these environments were associated with maximum g_s. These findings, combined with evidence from an earlier study (Powers et al., 2008b) suggest moderate to large canopy gaps may provide a regeneration environment that minimizes seedling moisture stress in northern pines while maximizing light availability. Second, our isotopic data suggests moderate partial canopies of approximately $10 \text{ m}^2 \text{ ha}^{-1}$ are associated with near-minimum E and maximum iWUE in pine seedlings. Basal areas of $13–14 \text{ m}^2 \text{ ha}^{-1}$ in northern pine forests are associated with light levels of approximately 50% full sun (Law et al., 1992; Wetzel and Burgess, 2001). These light levels promote nearly maximum growth rates in eastern white

pine seedlings (Burgess and Wetzel, 2000; Boucher et al., 2007) and moderate growth rates in red pine seedlings (Powers et al., 2008b). This suggests the overstory environments that minimize transpirational water losses and maximize seedling water use efficiency may also provide enough light for near-optimal to optimal growth of red and white pine regeneration, with the added benefit of increased residual tree growth (Zenner and Krueger, 2006).

5. Conclusion

Our results indicate pine seedling E declined across a gradient of overstory conditions ranging from open canopy gaps to closed canopy environments due to reductions in g_s rather than changes in evaporative demand. Intrinsic water use efficiency increased slightly from open gaps to partial canopy environments with moderate basal areas. These findings suggest moderate to large sized canopy gaps could provide the best environments for reducing seedling moisture stress in eastern North America's mixed jack, red, and white pine forests, while residual overstory basal areas of approximately $10 \text{ m}^2 \text{ ha}^{-1}$ could maximize tradeoffs between productivity and water loss.

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